EFFECTS OF FIELD STRENGTH AND IONIC STRENGTH ON VELOCITY AND SPREAD OF ZONES IN STARCH BLOCK ZONE ELECTROPHORESIS

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As has been implied by TISELIUS AND FLODIN[1] in their review, optimal conditions of field strength and ionic strength in zone electrophoresis represent compromises. This is further brought out in the present study in which the performance of a modified design of starch block zone electrophoresis apparatus was tested. Thus it will be shown that where high fields lead to high velocities, they also lead to undesirable generation of heat; and where the use of low ionic strengths counteracts generation of heat, it also results in disturbed zones. Studies of this nature are especially pertinent to the problem of whether the apparent demonstration by zone electrophoretic procedures of the occurrence of multiple components in enzymes, such as in cellulase[2,3], might be ascribable to artifacts arising from non-ideal experimental conditions.

APPARATUS

The principal parts of the zone electrophoresis apparatus used are shown in the photograph in Fig. 1. They consist of a base, A; electrode compartments, B; troughs, C, which contain the starch supporting medium; an interlock switch, D, controlled

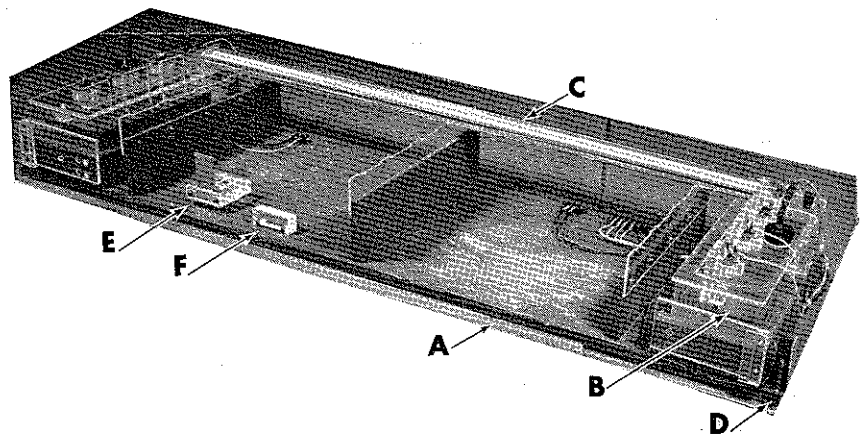

Fig. 1. Photograph of modified design of zone electrophoresis apparatus.

by a safety shield; a cutter, E, for sectioning starch blocks at the ends of runs; and a shaver, F, for trimming the surfaces of the blocks to a uniform level.

The troughs consist of Pyrex plates, 100 × 5.1 × 0.4 cm in dimensions, to which Plexiglas shoulders, 100 × 0.9 × 0.3 cm are cemented with the aid of Goodyear adhesive, Pliobond 30. Plexiglas was used for the shoulders in the belief that edge effects[4] might be reduced by virtue of the low heat conductivity of Plexiglas relative to Pyrex.

A buffer circulating system, a voltage monitoring system, a power supply and electrical distribution system, and a safety shield, also used, are not shown in the photograph. Further description of the apparatus and accessories is given elsewhere[5].

PROCEDURE

Approximately 110 g of potato starch granules were thoroughly mixed with about 90 ml of buffer. The proportion of buffer to starch was that which would make a slurry which was just loose enough to flow, yet not excessively wet. The ends of the trough were closed off with cellophane adhesive tape and the slurry was then poured into the trough. To remove air bubbles and to pack the starch evenly, the trough was agitated on a vibrating table. The excess buffer which rose to the surface was removed by blotting with folded sheets of filter paper, but care was taken not to remove more buffer than necessary. Excess starch was trimmed to within 3/128 inch of the level of the shoulders of the trough with the aid of the shaver. A wafer consisting of a mixture of 0.1 ml of buffered test sample with an appropriate quantity of starch granules was introduced into a slit prepared in the block midway between the ends. The block was then trimmed to within 1/128 inch of the shoulders, the top glass plate was placed on the block, and good contact was assured with the aid of 6 No. 18 ball joint clamps. The tape was removed from the ends of the block and the block was placed on the rack between the electrode compartments. Cotton cloth wicks were inserted between wick clamp plates and the ends of the block and the block was allowed to equilibrate for about 1 h before the desired voltage was applied. On completion of the run, the block was sectioned, the sections were mixed with definite volumes of water, and the resulting extracts were analyzed for components. Analyses for protein were made by a modification[6] of the method of LOWRY and for polysaccharide by the method of RIMINGTON[7].

EXPERIMENTAL

Tests of the performance of the modified starch block zone electrophoresis apparatus were first made using 0.1 ionic strength veronal buffer at pH 8.6, a field of 5 V/cm, and test samples consisting of mixtures of 5 mg of bovine plasma albumin, 10 mg of hemoglobin, and 5 mg of dextran. The bovine plasma albumin was obtained from the Armour Laboratories, Chicago, the hemoglobin by hemolysis of washed human red cells, and the dextran from E. T. REESE of these laboratories. All electrophoresis runs were carried out in a cold room at an ambient temperature of 2–4°. Separate runs

were made for periods of 2, 4, 8, 16 and 32 h. Visual inspection at the ends of the runs revealed that zones of the hemoglobin were quite even and did not show significant curvature or cometing. Patterns of protein and carbohydrate distribution obtained for the 4-h and 32-h runs are shown in Fig. 2. The progress of the resolution is shown diagrammatically in Fig. 3. The widths of the zones were determined by measuring

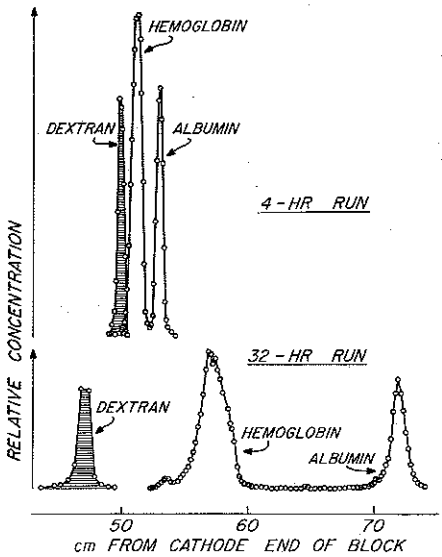

Fig. 2. Electrophoretic diagrams of mixtures of bovine plasma albumin, hemoglobin, and dextran obtained after different periods.

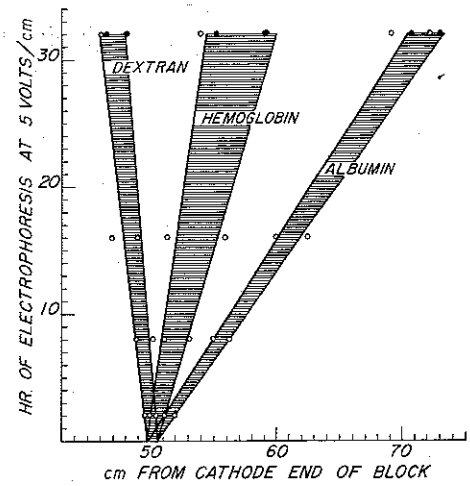

Fig. 3. Progress of resolution during electrophoresis of mixture of plasma albumin, hemoglobin, and dextran. Solid points represent results of duplicate measurements.

the distances between baseline intercepts of tangents drawn at inflection points on either side of the peaks. The results shown in the figures indicate relatively linear, disturbance-free migration with time.

Tests were next made to determine whether fields higher than 5 V/cm could be used and whether the expected adverse effects of heat generated at higher fields could be counteracted by the use of low ionic strengths. Fields of 5.0, 7.5 and 13 V/cm and ionic strengths of 0.05, 0.1 and 0.2 were tested, and all runs were made for 80 volt-hours/cm. At intervals during the runs, temperature measurements were made with the aid of thermocouples. Distances of migration, widths of zones, and temperatures of blocks at ends of the runs are summarized in Table I. Distances of migration for the dextran are related to the starting position of the initial zone; distances for the hemoglobin and albumin are related to the final position of the dextran. Also given are distances of migration corrected for viscosity of the buffer and for change of buffer viscosity resulting from change in temperature during the runs. The viscosity corrections are referred to water at 2.5°. Zones which were observed in the runs made at 13 V/cm with buffer of 0.2 ionic strength were too badly disturbed to merit measurement and are not, therefore, evaluated in Table I.

TABLE I

Field V/cm	Component	0.05 ionic strength				0.1 ionic strength				0.2 ionic strength			
		Dist. cm	Dist. corr.* cm	Width cm	Temp. °C	Dist. cm	Dist. corr. cm	Width cm	Temp. °C	Dist. cm	Dist. corr. cm	Width cm	Temp. °C
5.0	Dextran	— 1.37	— 1.36	1.27	3.7	— 1.26	— 1.27	1.23	4.3	— 1.58	— 1.62	1.21	5.1
	Hemoglobin	+ 5.82	+ 5.76	2.23		+ 5.35	+ 5.34	1.49		+ 4.70	+ 4.83	1.45	
	Albumin	+ 14.91	+ 14.75	1.63		+ 12.58	+ 12.56	1.57		+ 9.93	+ 10.19	1.40	
7.5	Dextran	— 1.72	— 1.64	1.48	4.9	— 1.32	— 1.27	1.35	5.6	— 0.25	— 0.24	1.35	7.1
	Hemoglobin	+ 6.34	+ 6.04	3.07		+ 5.85	+ 5.61	2.09		+ 4.60	+ 4.44	1.99	
	Albumin	+ 15.56	+ 14.82	1.75		+ 13.16	+ 12.61	1.43		+ 10.03	+ 9.67	1.53	
13	Dextran	— 1.98	— 1.60	1.83	10.9	— 2.39	— 1.61	1.52	19.4				40.4
	Hemoglobin	+ 7.32	+ 5.91	3.80		+ 7.71	+ 5.22	2.60					
	Albumin	+ 17.83	+ 14.40	2.14		+ 17.87	+ 12.10	2.16					

* Distances of migration corrected for viscosity of the buffer and for change of buffer viscosity resulting from change in temperature during the runs.

The data show, first of all, that distances of migration of hemoglobin and albumin, per 80 volt-hour/cm, increase with voltage and decrease with ionic strength. The increase with voltage is probably due, however, to the effect of generation of heat on viscosity of the buffer medium since temperature corrections for viscosity eliminate this effect. The decrease with ionic strength which was observed was also noted by TISELIUS AND FLODIN[1]. The distances of migration of the dextran are rather erratic and cannot be correlated well with voltage or ionic strength. This behavior of the dextran may be the result of variable movement of the starting zone during equilibration of the block, which precedes the application of the field.

The widths of the zones of all three test materials increase with voltage and decrease with ionic strength. Visual inspection of hemoglobin zones during runs made with high voltage or low ionic strength revealed marked irregularities in shape, suggesting electrical or mechanical disturbances within the block. Such disturbances presumably extend to some degree to the invisible zones of albumin and dextran. The disturbance with high voltage might be caused by non-uniformity in temperature, giving rise to uneven field, variable viscosity, and convection. Possible causes for disturbances with low ionic strength are not known. It should be pointed out, however, that the effect of ionic strength upon degree of spread is quite dependent upon the nature of the test material, that for hemoglobin being particularly marked, while those for albumin and dextran are very slight. This suggests that disturbances arising from low ionic strength may be more local than general.

Optimal results with the electrophoretic system used, in terms of narrowness and uniformity of zones, are obtained with fields of 7.5 V/cm or lower and with buffer media of 0.1 ionic strength or higher. Under such conditions the spread of albumin with time of migration is only slightly higher than that of dextran while that of hemoglobin is considerably greater. The spread of the hemoglobin peak reflects its known electrochemical inhomogeneity[8].

Distance of migration, *per se*, would not appear to be related to spread to a significant degree since, under favorable conditions of electrophoresis, zones of albumin after migration of 10–13 cm show but slightly greater spread than zones of dextran which have migrated only 0–2 cm. Neither does diffusion appear to be an important factor affecting spread since it was found in separate experiments that glucose and other low molecular weight uncharged materials, having relatively high diffusion rates, show approximately the same spread as does dextran. The possibility is suggested that the spread observed with an uncharged substance might be used as a basis for judging the heterogeneity of charged substances.

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SUMMARY

Fields that are too high or ionic strengths that are too low may cause disturbances in

starch block zone electrophoresis. With the apparatus used, optimal results were obtained with fields of 7.5 V/cm or lower and with ionic strengths of 0.1 or higher.

Spread of zones does not appear to be related to a significant degree to distance of migration, *per se*, or to diffusion. The possibility is suggested that the spread observed with an uncharged substance might be used as a basis for judging the heterogeneity of charged substances.

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